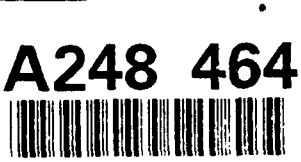
AD-A248 464



AGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE	3. REPORT TYPE AND DATES COVERED
	FINAL 15 Jun 90 TO 14 Dec 91

4. TITLE AND SUBTITLE
CUMULATIVE EFFECT OF REPEATED BRIEF CEREBRAL ISCHEMIA

5. FUNDING NUMBERS
G AFOSR-90-0269
PE 61102F
PR 2312
TA A5

6. AUTHOR(S)
Dr James H. Halsey, Jr

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Alabama at Birmingham
School of Medicine
Dept of Neurology
1210 Jefferson Tower, 619 19th Street South
Birmingham, AL 35294

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 92 0199

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Dr Cornette
AFOSR/NL
Building 410
Bolling AFB DC 20332-6448

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited.

12b. DISTRIBUTION CODE

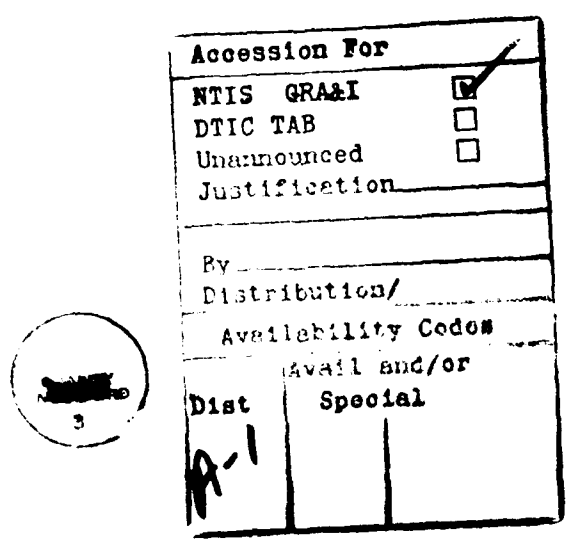
13. ABSTRACT *(Maximum 200 words)*

We have investigated the problem of Gz-induced blackout in an animal model in which controlled brief global cerebral ischemia is produced repeatedly at short intervals. The objective was to determine if this results in cumulative impairment of brain metabolism and electrical function and if so to identify the mechanisms involved. Our intial hypothesis was that accumulation of lactate may be an important element in this process. Rats were prepared for experiment under halothane anesthesia with tracheostomy, ligation of both subclavian arteries, cannulation of one femoral artery, and of one common carotied artery in cephalad direction. Both external carotid arteries were ligated. Both mouth and rectal temperatures were monitored with thermocouples and mouth terperature was maintained at 37.0±0.5 degrees Celsius. A balloon in cuff was placed around the remaining carotid artery. The rat was placed within the spectrometer magnet operating at 4.7 Telsa for lactate and phosphorous. Repeated brief episodes of global brain ischemia were made by temporary inflation of the carotid balloon while MR proton spectra are being acquired serially at intervals of 30 seconds using at 1 cm surface coil. We report results using a newly constructed doubly tuned probe enabling interleaved acquisition of 31P (high energy phosphates

14. SUBJECT TERMS	15. NUMBER OF PAGES
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

and pH) and 1H (lactate and amino acids). Different durations of ischemia and intervals between them were tested to determine thresholds for development of EEG impairment, tissue acidosis, high energy phosphate depletion, and lactate accumulation.



Final Technical Report

Cumulative Effect of Repeated Brief Cerebral Ischemia

ABSTRACT

We have investigated the problem of Gz-induced blackout in an animal model in which controlled brief global cerebral ischemia is produced repeatedly at short intervals. The objective was to determine if this results in cumulative impairment of brain metabolism and electrical function and if so to identify the mechanisms involved. Our intial hypothesis was that accumulation of lactate may be an important element in this process. Rats were prepared for experiment under halothane anesthesia with tracheostomy, ligation of both subclavian arteries, cannulation of one femoral artery, and of one common carotid artery in cephalad direction. Both external carotid arteries were ligated. Both mouth and rectal temperatures were monitored with thermocouples and mouth temperature was maintained at 37.0±0.5 degrees Celsius. A balloon in cuff was placed around the remaining carotid artery. The rat was placed within the spectrometer magnet operating at 4.7 Telsa for lactate and phosphorous. Repeated brief episodes of global brain ischemia were made by temporary inflation of the carotid balloon while MR proton spectra are being acquired serially at intervals of 30 seconds using a 1 cm surface coil. We report results using a newly constructed doubly tuned probe enabling interleaved acquisition of 31P (high energy phosphates and pH) and 1H (lactate and amino acids). Different durations of ischemia and intervals between them were tested to determine thresholds for development of EEG impairment, tissue acidosis, high energy phosphate depletion, and lactate accumulation.

This report is submitted now after completion of one years work. Preliminary results indicate the desirability of continuing the work. Two additional years of funding are requested, to begin hopefully by June 1 1992, anticipating a 6 month hiatus in funding December-June 1992.

INTRODUCTION: Pilots of high performance aircraft are subject to transient loss of consciousness due to sudden high gravitational stress. This is presumed to be due to cerebral ischemia resulting from failure of blood delivery to the brain due to the severe gravitational stress. This has been confirmed by Transcranial Doppler monitoring in volunteers in centrifuge (Wersham, personal communication 1989).

GENERAL AIM: To determine if repeated brief cerebral ischemia, as occurs in Gz induced "blackout" can cause cumulative functional and structural brain damage, and if so to determine if this can be ameliorated or prevented.

HYPOTHESIS: Repeated brief episodes of ischemia produce cumulative impairment of function and metabolism, manifested by progressive loss of EEG, increasingly delayed EEG recovery, and accumulation of lactate with depletion of high energy phosphates and development of acidosis. If sufficiently severe this may result in neuronal death or cerebral infarction. Determinants of ultimate outcome include the residual cerebral perfusion pressure during ischemia, and its level during reflow, the relative durations of ischemia and reflow, and the number of repetitions. The amount of lactate accumulating, influenced by blood glucose and perhaps other factors including lactate transport, may be an additional determinant. The interaction of these determinants will be reflected in the accumulation of cerebral lactate concentrations, the depletion of high energy phosphates and brain tissue acidosis.

SPECIFIC AIMS:

1. By means of simultaneous 1H and 31P MR Spectroscopy to monitor lactate accumulation, amino acid depletion, acidosis, and high energy phosphate depletion during standardized periods of repeated brief ischemia in a rat model in which cerebral perfusion pressure is monitored and controlled, and EEG monitored.

2. To determine the thresholds at which these variables in different combination result in disappearance of EEG, delay or prevent its recovery, and result in histologically detectable brain tissue damage.

RATIONALE: Repeated brief cerebral ischemia presents the special opportunity to study **graded** delivery of ischemic insult over a specified time period, in contrast to the classical situation of acute stroke in which an all-or none insult is delivered immediately. Magnetic Resonance Spectroscopy (MRS) is an ideal method for monitoring this evolution by in vivo serial measurements. The method however is complicated by severe logistic constraints which must be mastered to enable collection of significant results.

RESULTS:

1. PERFECTION OF THE RAT MODEL

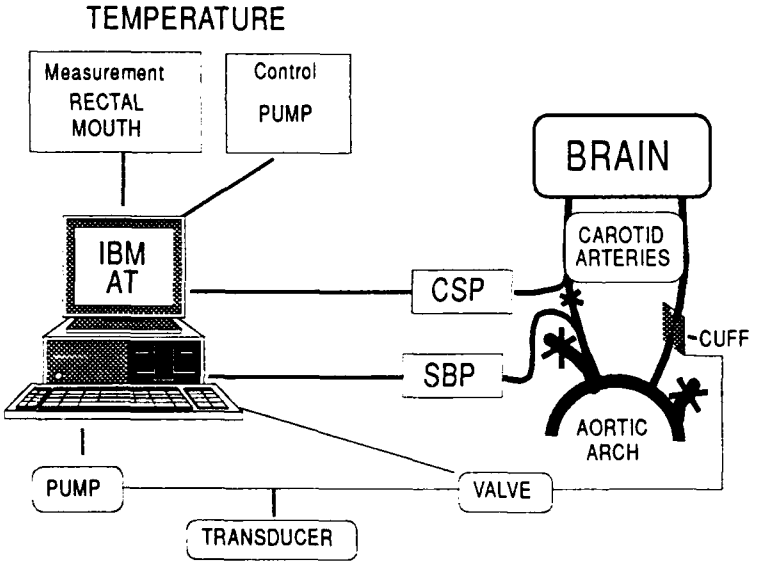


Figure 1. Block diagram of the components in the automatic occluder system. The IBM-AT at the core of the system monitors carotid and systemic blood pressures and maintains a constant pressure occluder system, which is under software control. In addition the system monitors mouth and rectal temperatures and controls the pumping of warm water through the probe to maintain mouth temperature at 37 degrees centrigrade.

Our laboratory has developed and extensively analyzed an animal model of global ischemia which can be partial or total, asymmetric or homogeneous, in which quantitative control of cerebral blood flow during ischemia and reperfusion is possible. The basic preparation is the rat with bilateral subclavian artery ligation proximal to the vertebral artery origins, with cannulation distally of one carotid artery in order to monitor the distal carotid stump pressure as an index of cerebral perfusion pressure. Total global ischemia results if the remaining carotid is occluded. This can be done repetitively at consistent intervals under computer control utilizing a pump activated extravascular balloon occluder (fig.1). Modulation of the systemic arterial blood pressure enables control of the flow during reperfusion (1).

2. THE EFFECT OF REPEATED BRIEF CEREBRAL ISCHEMIA ON EEG.

This preliminary study was performed to test the hypothesis that repeated episodes of brief cerebral ischemia cause cumulative brain dysfunction. This was done as a preliminary assessment of the animal model, for protocol development, and to assess data from another laboratory suggesting that in fact progressive metabolic **adaptation** occurred. We monitored EEG as the primary index of brain function/metabolism, and cerebral cortical PO2 as an index of blood supply, in addition to the right carotid artery stump pressure as an index of cerebral perfusion pressure at the level of the circle of Willis.

Our data suggest that metabolic **deterioration** does occur, but may be countered by improved blood supply. Amelioration was always associated with improving brain perfusion pressure.

Oxygen Electrodes: Electrodes were manufactured from 90% platinum 10% iridium wire of 100 microns diameter and 1mm exposed tip length. Under halothane anesthesia, two were implanted through small cranial burr holes with the tips approximately 1.5 mm deep into the cortex of each hemisphere and fixed in place with dental acrylic cement. A single silver-silver chloride reference electrode was implanted subcutaneously.

The rats recovered from anesthesia and were maintained 3-5 days to enable healing of the capillary circulation and formation of a glial membrane around the electrodes. We have analyzed this electrode-tissue relationship, and demonstrated that the recorded current is proportional to local tissue PO2, hence the term PO2%. The volume of tissue represented in the measurement is a function of the electrode diameter. For our electrodes 1mm in length and 100 microns in diameter this is a cylinder with a radius of about 500 microns surrounding the electrode. A given electrode, chronically implanted, provides semiquantitative reliability for demonstrating changes in tissue PO2% and can be monitored continuously (2). To minimize a contribution to the recording from electroreduction of halothane a polarization voltage of -500 mV was used. At this voltage, anesthetic levels of halothane contributed 1-2% of the recorded current otherwise attributed to oxygen.

Surgical preparation, production of repeated cerebral ischemia, control of brain temperature, and monitoring of EEG were generally as described below in the METHODS OF PROCEDURE section.

In these experiments the duration of ischemia was 20-40 seconds followed by reperfusion for the remainder of the minute such that each cycle was 60 seconds in duration, ie 20 seconds ischemia-40 seconds reperfusion, 30 seconds ischemia-30 seconds reperfusion. The duration of ischemia and reperfusion was constant for each experiment which consisted of 30 successive cycles over 30 minutes. The computer simultaneously monitored EEG, carotid stump and systemic arterial blood pressure.

Results: Six rats were thus studied. Each hemisphere was considered independently. In one rat since no significant EEG abnormality was present one hour after completion of the 30 cycles of ischemia-reperfusion, a second set of cycles was performed. This yielded 14 sets of EEG, PO2, and CSP recordings for analysis. The PO2 for the two electrodes in each hemisphere was averaged to facilitate the correlation with the EEG which was derived between them.

Generally, upon occlusion of the left carotid artery the CSP fell to 6-8 mm Hg. The systemic blood pressure displayed a Cushing response, reflecting the cerebral ischemia, rising rapidly from a non ischemic level of around 100 mm Hg to 160-180 mm Hg. In some rats this caused slight recovery of the CSP to 10-12 mm Hg near the end of the occluded interval.

The PO2 fell rapidly, to less than 30 per cent of the non ischemic level, within about 10 seconds, and then more slowly. In some rats it did not reach zero within the brief period of ischemia, though it would reach zero after a longer interval. EEG disappeared within about 15 seconds. This probably reflected minor residual flow. Upon release of the occlusion the PO2 rose rapidly in both hemispheres, and EEG began to recover. Fast activity was more sensitively affected by ischemia than slow activity and was slower to recover during reperfusion.

In pilot studies, we found that periods of ischemia less than 20 seconds with reperfusion longer than 40 seconds generally resulted in complete EEG recovery during each reperfusion with no tendency for the EEG to deteriorate with successive cycles. With longer ischemia and shorter reperfusion EEG recovery became progressively less complete with successive cycles of ischemia. For the formal analysis below, the cycle durations were ischemia 20 seconds reperfusion 40 seconds for two hemispheres, 30 seconds ischemia 30 seconds reperfusion for 2, 35-25 for 6, and 40-20 for 4.

For each hemisphere, from the continuously monitored PO2 (average of the two electrodes), EEG fast activity, and right carotid artery stump pressure, the following tabulations were made and analyzed, comparing the values for the first cycle of ischemia-reperfusion with the 30th for the average tissue PO2 1 second prior to onset of reperfusion, carotid stump pressure 1 second prior to onset of reperfusion, average amplitude of EEG fast activity during the last 10 seconds of reperfusion. To facilitate the comparisons, each EEG and PO2 value was expressed as a per cent of its control value prior to the first episode of ischemia. The CSP changes were in mm Hg.

	First cycle	Last Cycle	Avg Change
CSP	9.9(3.0=s.d.)	9.8 (1.9)	-0.07
PO2	10.0 (5.3)	10.2 (4.4)	0.21
EEG	30.2 (18.4)	20.6 (18.3)	-9.5

The degree of EEG recovery progressively declined from first reperfusion to last in 11 hemispheres and improved in only 3. Both the PO2 and the CSP showed increases during the successive cycles in those 3, suggesting that these instances of EEG improvement depended upon blood flow adaptation rather than on neuronal metabolic adaptation. Moreover the stronger correlation between CSP and PO2 suggested that most of the blood flow adaptation was of the extracranial collateral, resulting in improved CSP, mostly due to the rise in systemic BP. Over all the correlation between CSP change and EEG change and between PO2 change and EEG change was very weak while if those 3 hemispheres showing EEG improvement were excluded then the correlations between EEG change and the two indices of flow were not significant, the EEG tending to deteriorate whether PO2 or CSP changed or not.

There was no relation between duration of ischemia (number of seconds in a 60 second cycle) and change in CSP or PO2 but as duration of ischemia was increased there was progressively more EEG deterioration.

Conclusion: These preliminary data suggest that progressive EEG deterioration, presumably reflecting progressive neuronal metabolic deterioration, occurs as a result of repeated episodes of brief cerebral ischemia. EEG recovery occurred only in the presence of recovery of blood supply and then inconsistently. Even in the presence of improving blood supply EEG deteriorated more often than it improved. Monitoring of carotid stump pressure was adequate to detect the improved blood supply if it was monitored accurately. Only a few mm Hg recovery could make a significant difference. Most of the blood supply recovery was attributable to the systemic arterial blood pressure Cushing response to the cerebral ischemia.

3. DETERMINATION OF LACTATE AND EEG IN THE 4.7 TELSA MAGNET (RAT IN VERTICAL POSTURE)

Since the beginning of funding we have constructed and tested a probe capable of proton spectroscopy, primarily for the purpose of monitoring relative changes in lactate concentration. Sensitivity is quite high, allowing acquisition of good quality spectra within 30 seconds, enabling comparison of lactate concentrations during individual brief occlusions and reperfusions.

Serial spectra have thus been acquired in four rats subjected to 30 successive 30 second occlusions with 30 second reperfusions, followed by one hour of uninterrupted reflow. We have the following preliminary observations:

1. Significant lactate accumulation occurred in each instance, reaching maximum levels 8-20 mM above control after about 10 successive occlusions, thereafter remaining relatively constant until cessation of the occlusions. At that point, the lactate rapidly cleared, with EEG recovery, within about 10 minutes in 3 of the 4 but did not clear in the fourth due to hypotension. We presume infarction occurred in that animal but have not yet performed the histology. Our experience with other animals under these conditions is that there would be no histologic abnormality in the 3 with prompt lactate clearance and EEG recovery.

2. In none of the four rats was there any depletion of amino acids despite isoelectric EEG, including the one which did not recover.

3. There was less than 10% difference in lactate concentration between occlusion periods and reflow (occlusion higher) during the first 10 successive occlusions and thereafter there was no difference.

HIGH MORTALITY DUE TO VERTICAL POSITIONING OF RAT

These experiments were frustrated by an extraordinarily high death rate due to progressively refractory postural hypotension. This is mainly a consequence of the vertical position in which the rat must be maintained because of the vertical bore of the magnet. It is probable that repeated occlusions are more stressful for the animal than a single occlusion, and in addition, the maximum rat size which could be accommodated in the probe (<200 gm) resulted in more fragile animals than we had used in the EEG study, and in our prior spectroscopy with sustained ischemia and hypoglycemia. The smaller animals in these experiments were a consequence of redesign of the probe to achieve the desired increased sensitivity (spectral acquisition in less than 30 seconds).

We obtained access to a horizontal magnet, 4.2 Tesla, with 40 cm bore which eliminates these constraints. Although spectral acquisition was slower (about 2 minutes) at this lower field strength compared with 8.5 Tesla in the narrow bore upright magnet, our preliminary

results confirm the feasibility of averaging periods of reflow and ischemia. The compelling reason to accept this lower field is to achieve tolerable experimental mortality less than 10%.

We subsequently moved to the new magnet, and using a newly constructed doubly tuned probe acquired interleaved 31P (high energy phosphates and pH) and 1H (lactate and amino acids).

4. ACQUISITION OF INTERLEAVED HIGH ENERGY PHOSPHATES AND LACTATE IN THE 4.2 TELSA MAGNET (RAT IN PRONE POSITION)

I am pleased at last to report technical success in simultaneous monitoring of 31P (ATP,PCr) and 1H (lactate). It proved enormously logistically complicated to move the experiments from the vertical narrow bore magnet to the horizontal large bore magnet. This was necessitated because the rats would not stay alive long enough under the postural stress of the vertical position in the face of the additional stress of the repeated ischemias. The complexity of the present arrangement results from the need for the respirator to be 15 feet from the magnet, in a separate room where the preparative surgery is carried out. The rat is then transported to the magnet, using a separate temporary respirator, placed in the magnet and reconnected to the original respirator. I'm sure you can imagine the number of separate steps involved, with the potential for each of them going wrong, as they did, beyond ordinary surgical morbidity and electronic recording problems. All have finally been mastered, and we are hopeful that the mastery will be maintained.

As you may remember, the rat was prepared by ligation of both subclavian and external carotid arteries. The right carotid was cannulated distally for monitoring of carotid stump pressure (reflecting cerebral perfusion pressure at the circle of Willis). Systemic arterial pressure was monitored and rectal and mouth temperatures were monitored and controlled at 37oC +/- about .5 degrees. An occlusive cuff was placed around the remaining left carotid (see figure 1 above).

The rat, under halothane+N2O anesthesia was thus subjected to repeated occlusions of the left carotid by inflating the cuff (near total ischemia) for a fraction of a minute followed by deflation (reflow) for the remainder of the minute representing one cycle of ischemia-reflow.

We have completed 3 successful experiments with simultaneous lactate (proton) and high energy phosphorous monitoring. Enclosed are charts showing the serial changes in lactate, PCr, ATP, and inorganic phosphorous (PI) during 4 or 5 series of repeated brief ischemias, 30 episodes in each series, each ischemia some fraction of a minute, eg 20:40 meaning 20 seconds ischemia, 40 seconds reperfusion. The time course of carotid stump pressure for each series is on a separate sheet for each experiment.

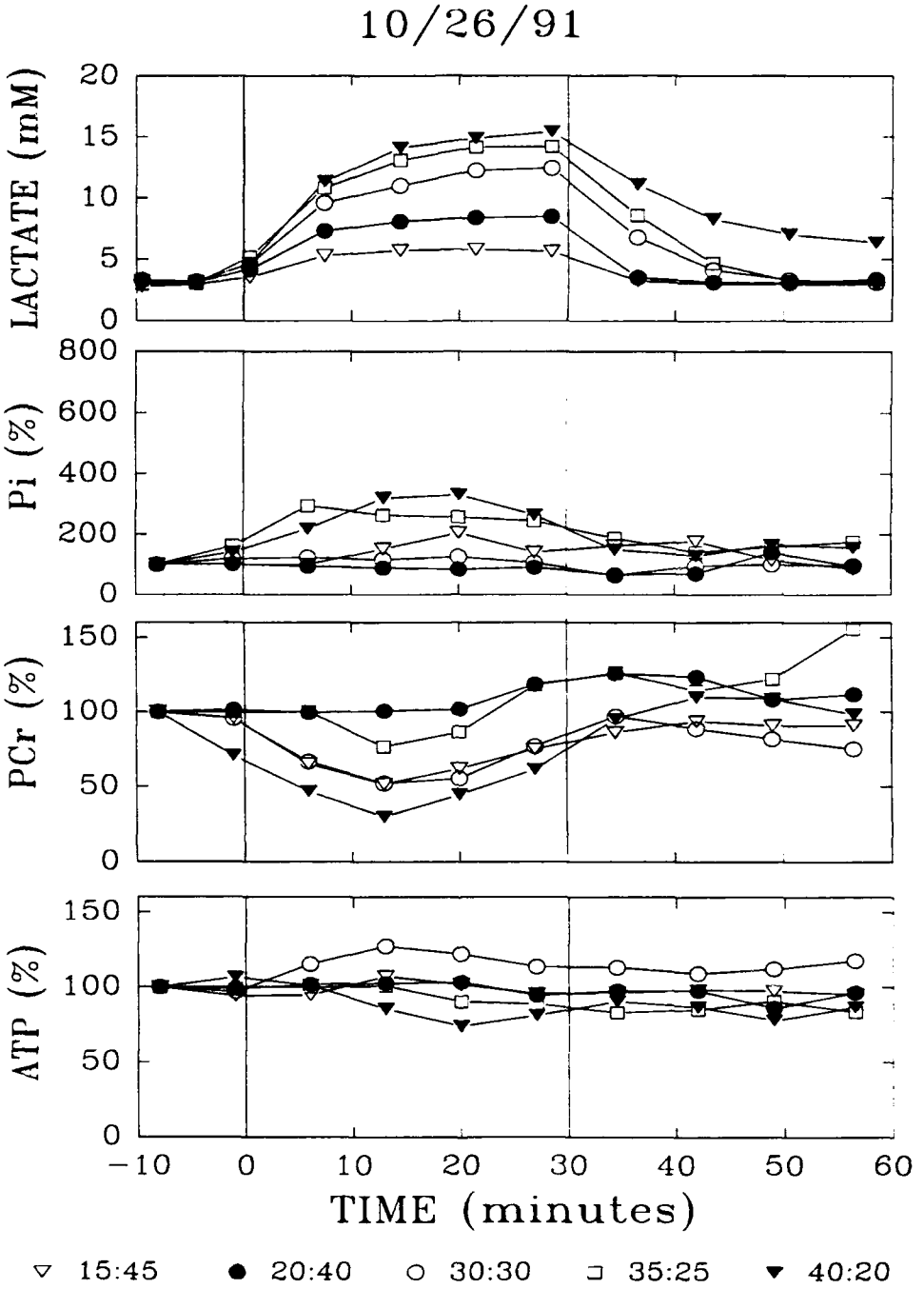


Figure 2. Lactate accumulation did not occur at 10/50 but was noticeable at 15/45 and more severe with each more severe insult. PCr depletion did not appear until near the end of the 30/30 series and appeared earlier in each successively more severe series. ATP depletion was only evident at the end of the 40/20 series. Lactate clearance was delayed after the 40/20 series but was prompt with those less severe. 30 such cycles represented one series. Six such series were performed, with periods of 20-40 minute rest periods of continuous reflow without ischemia, continued until the spectra normalized: 10 sec ischemia/50 sec reflow, 15/45, 25/35, 30/30, 35/25, and 40/20. The actual sequence in this experiment was 30/30, 10/50, 15/45, 20/40, 25/35, and 20/40.

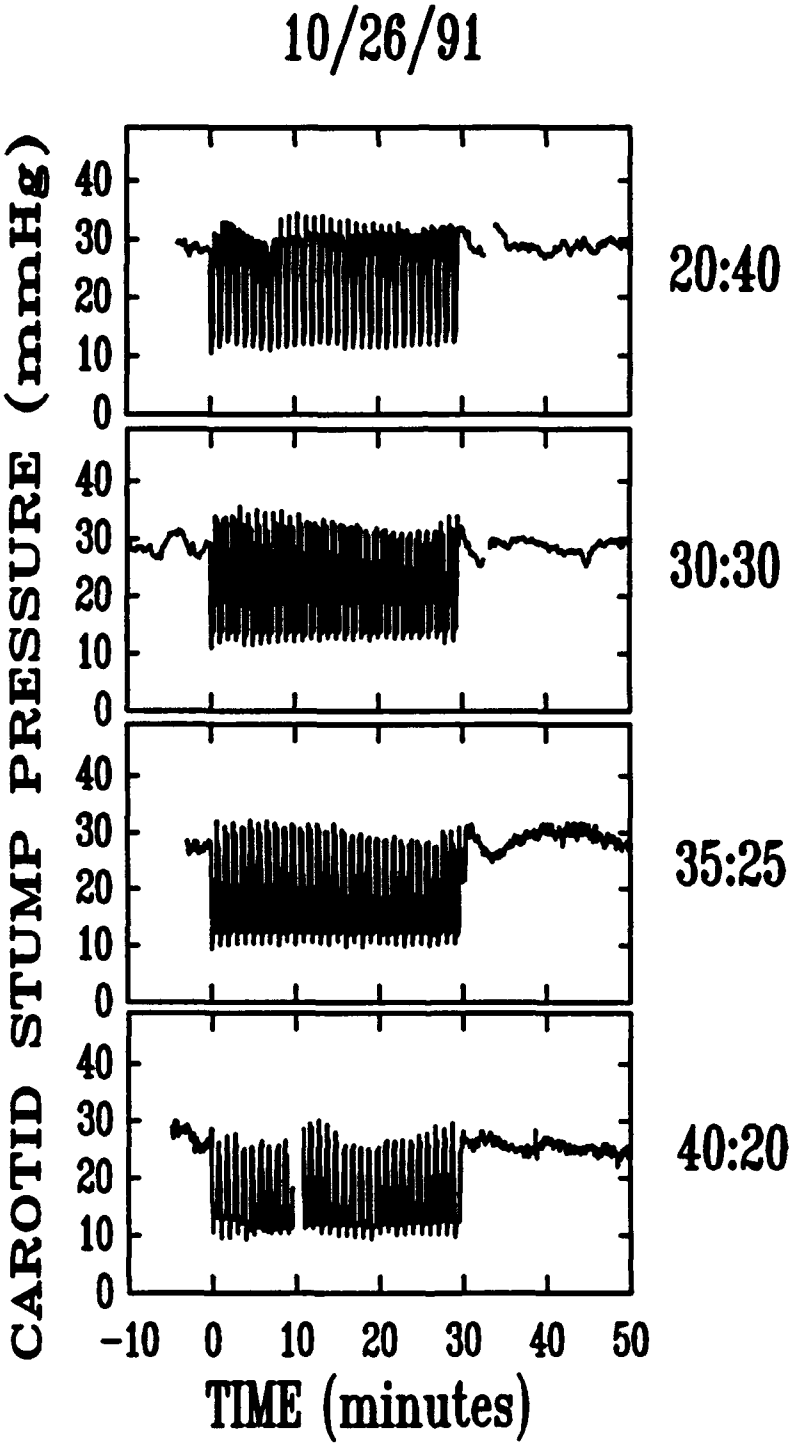


Figure 3. Carotid pressures measured during occlusion in the same whose metabolites are depicted in figure 2. Note consistent reduction of pressure to approximately 10 mmHg.

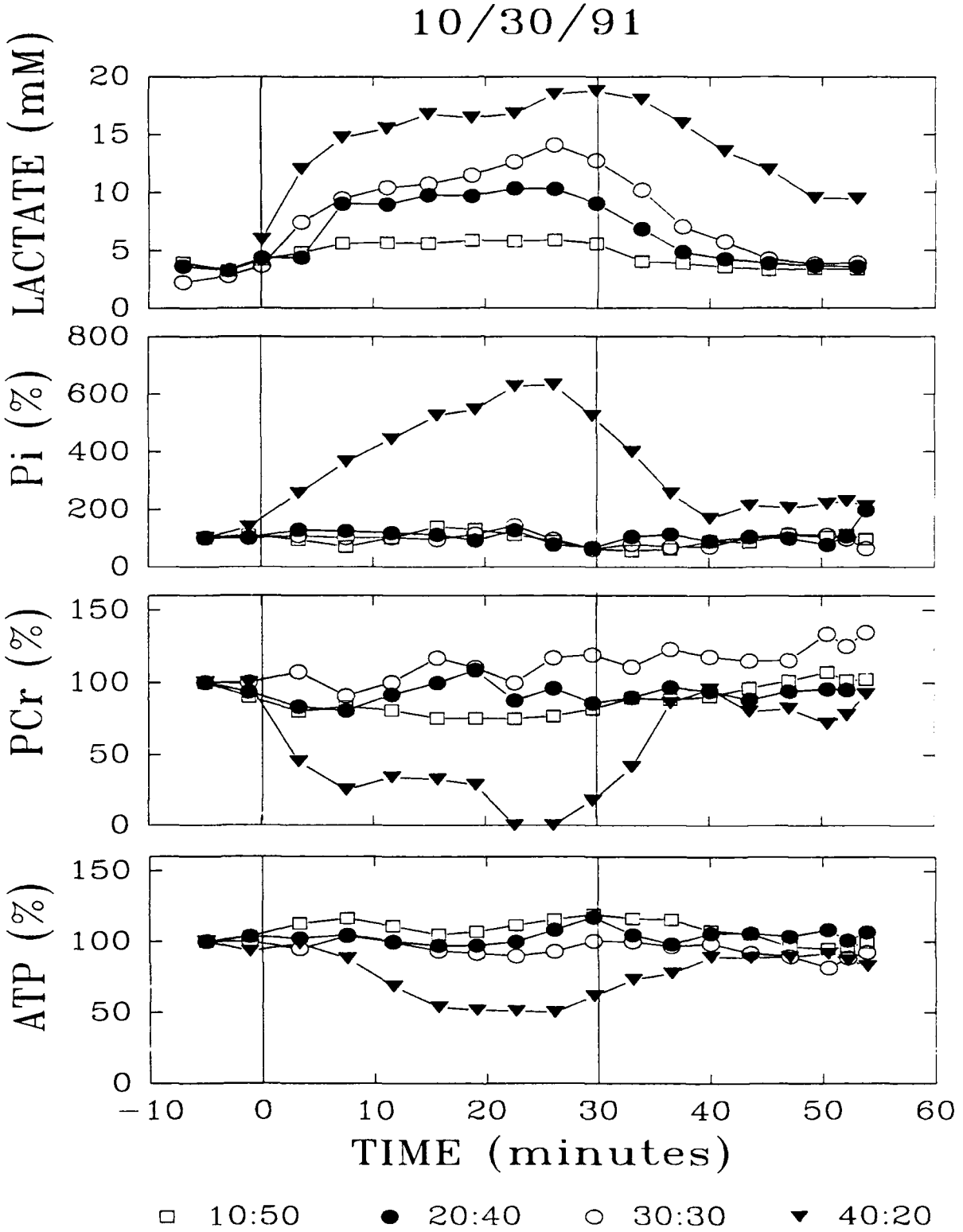


Figure 4. Lactate accumulation is apparent in all occlusive cycles. There is a pronouced further rise in lactate toward the end of both 30:30 and 40:20 cycles, which is easily correlated with a further drop in the carotid stump pressure at these times(see fig 5). This pressure drop further correlates with the collapse of PCr late in the 40:20 cycle.

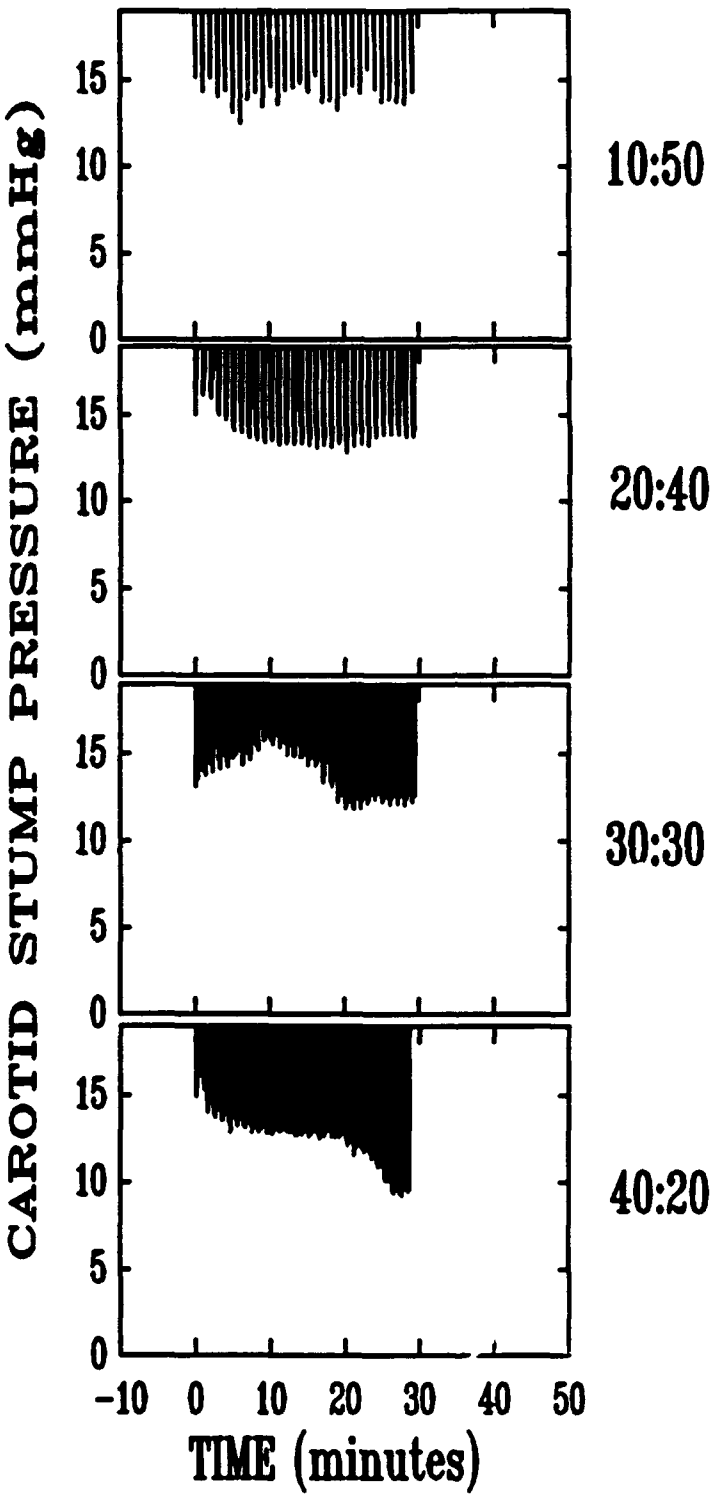


Figure 5. Plot of carotid pressure changes found in the 10/30/91 animal (fig. 4), plotted to emphasize the drop in pressure found at the end of the two most severe ischemic cycles.

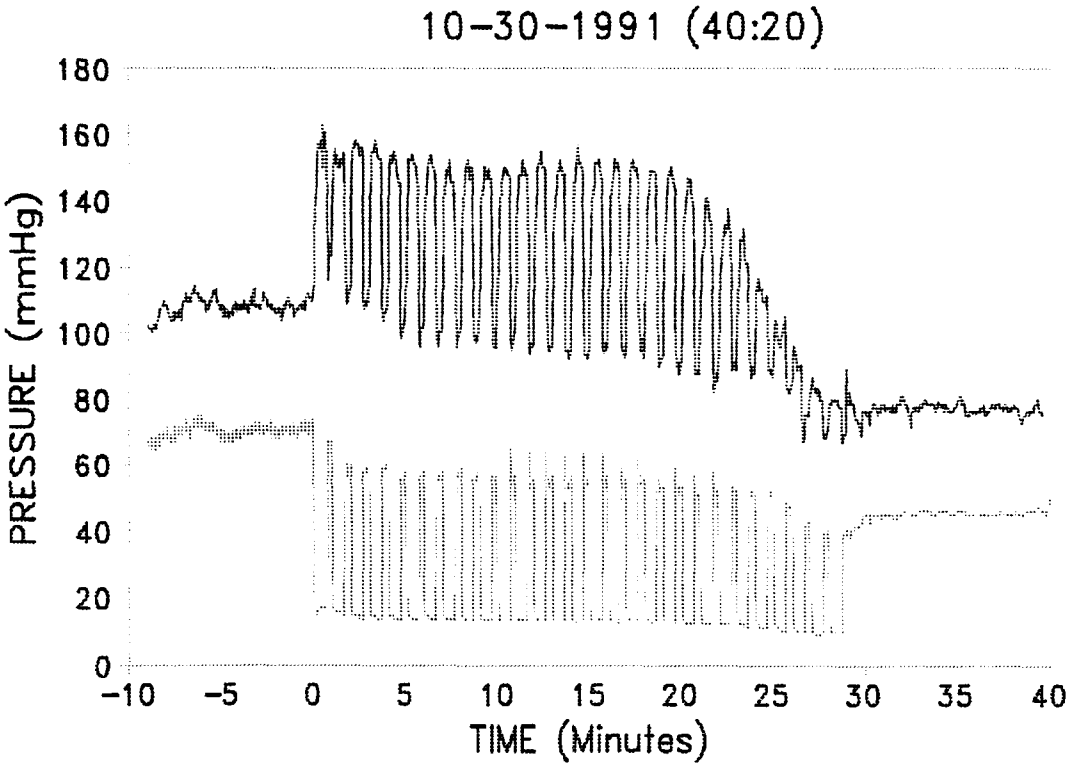


Figure 6. Plot of systemic and carotid pressures during 40:20 occlusive series. Note loss of Cushing response coincident with the collapse of PCr (fig. 4). Collapse of systemic pressure seemed imminent therefore this experimental cycle was terminated following 29 cycles.

The PCr and ATP determinations are less accurate than the lactate because of poorer signal:noise discrimination in the phosphorous spectrum. However the qualitative relation is clear that lactate is more sensitive than PCr, which is more sensitive than ATP, consistant with what has been seen in hypoxia, ordinary ischemia, and hypoglycemia. We also know that EEG is more sensitive than PCr, while neuron damage is not to be expected until after severe ATP depletion has occurred.

We are pushing forward to improve the signal:noise in the phosphorous spectrum. At present the switching from Phosphorous detection to protons has to be done manually by unplugging and replacing cables with large opportunities for errors and some noise generation. Hopefully automatic switching can soon be implemeted to take care of these, and improved pulse sequences will effect further improvement. An external reference to permit more accurate calibration of the PCr and ATP changes will be added.

We plan more experiments like this one to confirm the threshold ranges. Quantitatively I expect that these will be influenced not only by the relative durations of ischemia and reflow, but also on the carotid perfusion pressure during ischemia (if not 0) and during reflow.

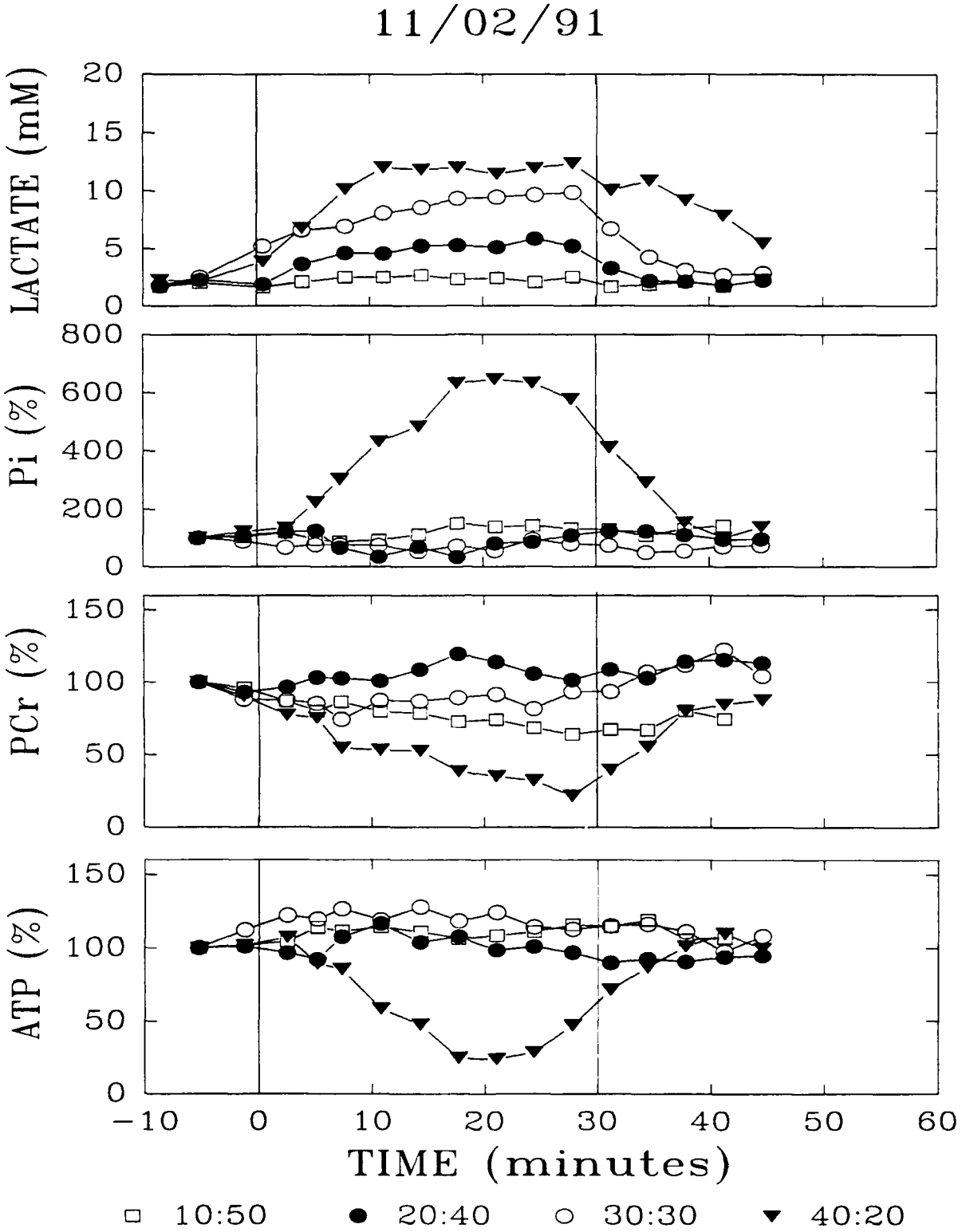


Figure 7. Although this animal accumulates less lactate than shown in figure 2, there is a much more marked reduction of ATP in this animal. Therefore, it seems unlikely that there is a direct correlation between lactate and energy loss in this model. It is more likely that metabolic rates and glucose reserves (to include glycogen) determine the succeptability of each animal to energy collapse.

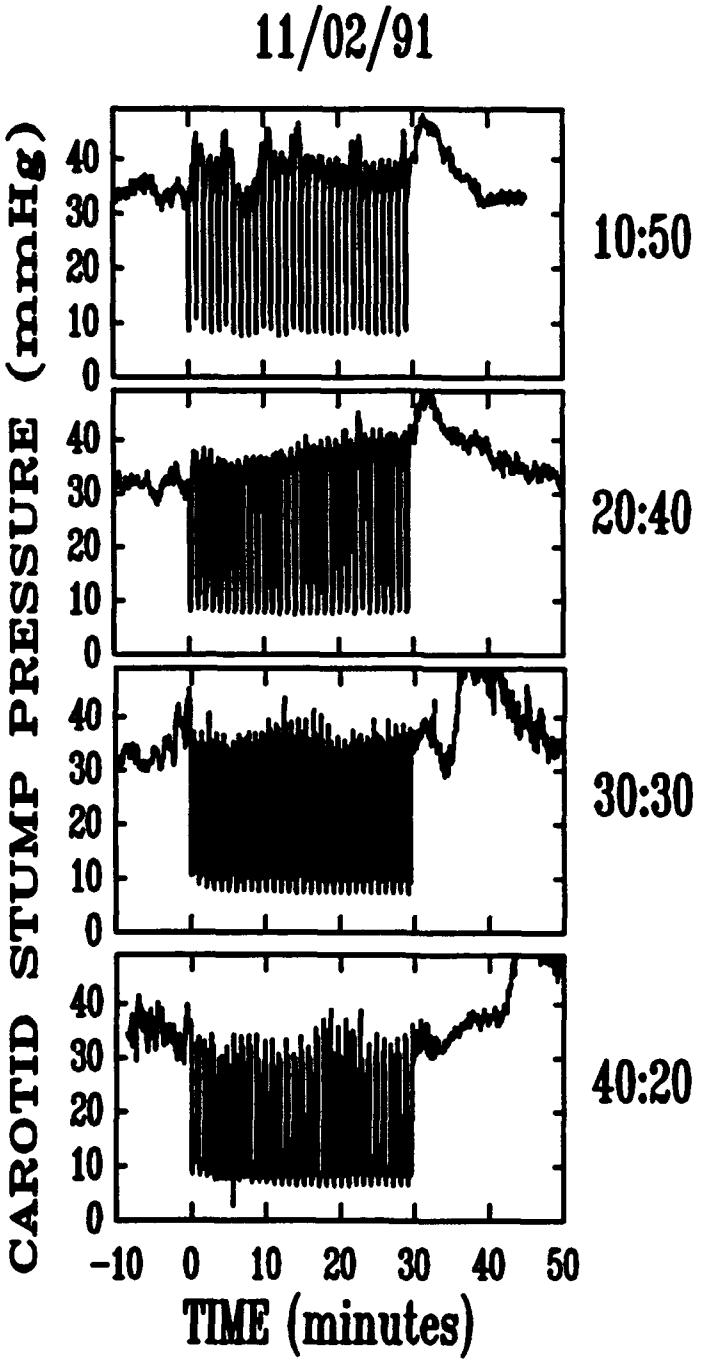


Figure 8. Carotid pressures measured during occlusion in the same whose metabolites are depicted in figure 7. Note consistent reduction of pressure to approximately 10 mmHg as was shown in figure 3.

An interesting feature of this preparation is that it has all the metabolic features of acute total ischemia, but these can be made to evolve slowly, in controlled fashion. A particular opportunity to explore is whether there might be adaptive changes in the rate of glycolysis and/or of Tricarboxylic

Acid cycle. This can be done with 13C labeled glucose, monitoring for appearance of the label in lactate and glutamate respectively. One of our collaborators here has experience with this measurement, including the necessary calculations.

It is clear that lactate is very sensitive to very mild ischemia, while only at severe ischemia is there any depletion of PCr, and even more severe is required to produce ATP depletion.In the absence of ATP depletion substantial neuronal necrosis is unlikely to occur, though minor (?reversible) structural changes could not be ruled out.

It is not clear if the lactate reaches a steady state plateau or continues to rise. We plan some 1 hour series to evaluate this question. There remains a good deal of noise in our phosphorous spectra. In the 10/26/91 experiment these were based on 4 minute accumulations, the last two 8 minutes which improved signal/noise somewhat. We plan 16 minute averages for the 60 minute experiments, anticipating a further improvement in resolution. By contrast we get very good lactate resolution with just one minute accumulation.

The poor s/n in the phosphorous accounts for the randomness in the changes at different severities less than the most severe (40:20) which consistantly showed considerable hydrolysis of high energy into inorganic phosphorous in all three experiments.

5. PRELIMINARY DATA FITTING AND MODELING OF LACTATE ACCUMULATION

A preliminary approach to evaluation of primary factors affecting rates of lactate accumulation and loss during intermittent ischemia and reperfusion.

Summary of modeling results: 1) Quantitative evaluation of the fractional occlusion time demonstrated that this variable was largely responsible for the determined lactate concentrations. 2) Quantitative evaluation of carotid stump pressures has shown a significant relationship between this parameter and the rates of lactate generation. 3) Temperature has not as yet been added to the model, but a strong linear correlation (r=.98) between maximum lactate concentrations generated by 40 second occlusions in three animals suggests that this variable may be a contributary cause of observed differences between animals. It is well documented that the enzymatic rates in the glycolytic pathway are temperature sensitive (see ref. 4 for a compilation of temperature coefficients for the glycolytic enzymes)

Our current conceptual state: We believe that the occlusive model functions as follows: With each occlusion lactate is generated linearly (3) largely as a function of the maximum catalytic rate of the glycolytic pathway. Glucose is not exhausted during the short occlusive process, and is subsequently replenished in the short reperfusion periods following occlusion therefore, absolute glucose brain or plasma levels do not affect lactate levels. Lactate concentrations reach plateau levels because lactate clearance from the brain is an exponential function of the brain lactate concentration. **Reservation**: Although there is sufficient data to suggest that glucose is not limiting with up to 30 seconds of occlusion, all 40 second occlusions were run as a terminal sequence. Hence, all 40:20 occlusions were performed at normo-glycemic or slightly hypoglycemic conditions. Therefore, the strong possiblity that absolute brain levels of glucose may affect thresholds of energy collapse should not be discarded, but rather should be investigated in future experiments.

To approximate the experimental results the following relationships were held to be true:

1) Lactate is generated at a constant rate during any short occlusive interval (3) (if occlusive pressure and temperature remain constant), (analysis of plasma glucose suggests that lactate generation is independent of this variable) thus;

Delta Lactate generation =
(a constant times the fractional occlusion time
times (a function of the carotid stump pressure)
times (a temperature function)

2) Lactate loss rate is a function of both the lactate concentration and the fractional reperfusion time, where the fractional reperfusion time is one minus the fractional occlusion time.

Thus;
Delta Lactate Loss = FracRepTime X Lactate loss rate

Washout of lactate is satisfactorily described by a single mono-exponential decay equation which applies to all animals. Thus, the rate of lactate loss is a linear function of lactate concentration. This result is consistent with carrier mediated transport of lactate through the blood brain barrier in these animals. The maximum rate of efflux of lactate from the brain has been estimated at 150 nmoles/min/g (5). Our rate of 143 nmoles/min/g agrees closely with their estimate. As these in vivo rates of efflux approximate the Vmax 120/nmoles/min/g of lactate transport across the blood brain barrier (6), it has been suggested (7) that the carrier mediated transport of lactate may be the limiting factor responsible for the observed rapid build-up of lactate during hypoxemia and the slow removal of lactate in post-hypoxemic recovery (5). Both starvation (8,9) and fat feeding (9,10) result in a slow progressive activation of the monocarboxilic acid transport system, thus on would expect the rate constant to change under these conditions.

For each cycle the current lactate concentration is of course the previous lactate concentration augmented by the lactate generated by the last occlusion and decreased by the amount of lactate removed during the subsequent fractional reperfusion time.

Preliminary Modeling of Lactate generation and loss assuming constant occlusion pressure and temperature.

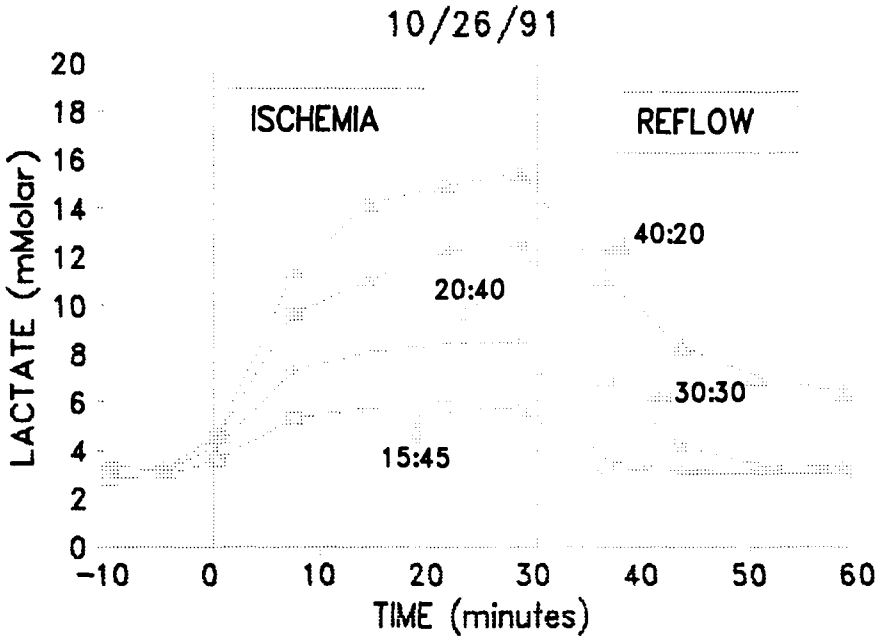


Figure 9. Plot of lactate versus time. Details as given under figure 2.

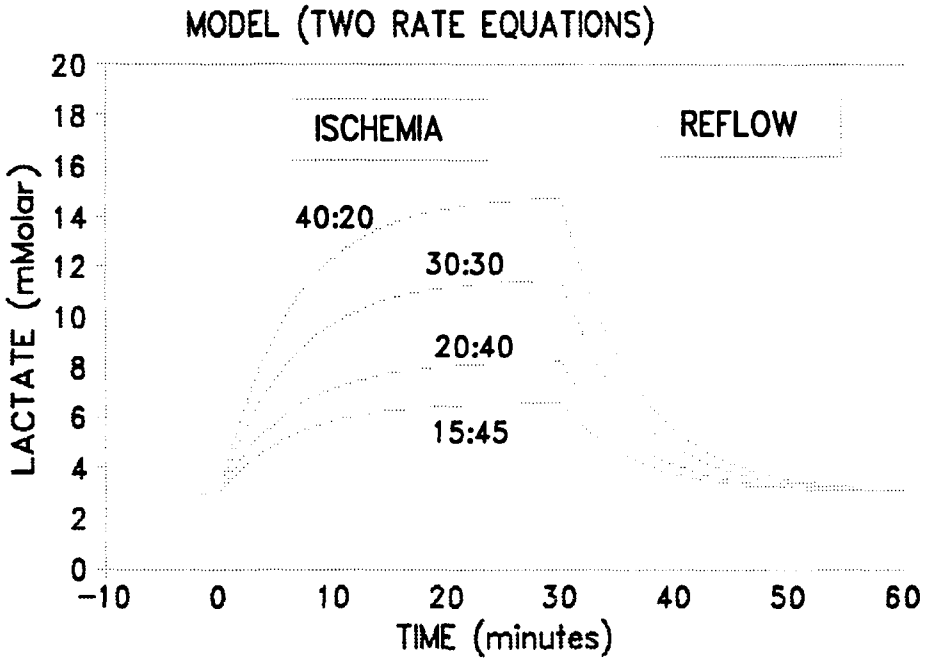


Figure 10 illustrates the preliminary non-optimized results of simulating the same experiment (lactate generations was assumed to be 3.3 mM/min).

Figure 9 above shows the experimental time course for 4 different duty cycles in the experimental animal from 10/26/91, while, figure 10 illustrates the preliminary non-optimized results of simulating the same experiment (lactate generations was assumed to be 3.3 mM/min.

Preliminary consideration of temperature changes present in the model:

Evaluation of temperature differences between animals suggests that this variable should be considered as a partial cause of observed differences between animals which were subjected to identical occlusive protocols.

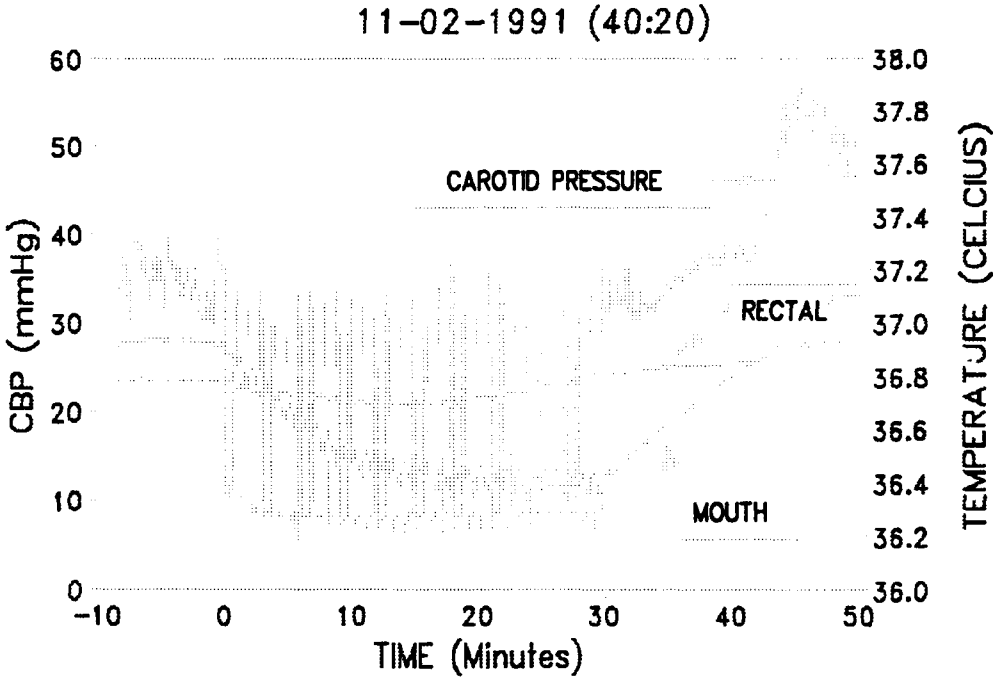


Figure 11. Within animal temperature changes also occurred. Typically, as is illustrated in figure 10, small mouth temperature (and presumably brain temperature) drops occur coincident with each occlusion. Thus, one might expect some temperature dependent decrease in metabolic rates of lactate generation coincident with this falling temperature.

METHODS OF PROCEDURE

Surgical preparation: The rats are anesthetized with 70% N2O, 29% O_2, and 1% Halothane, and atropine sulfate 0.004 mg/100 gram body weight injected. Tracheostomy is made, and the rat paralyzed with tubocurarine chloride and mechanically ventilated. Injections of atropine and tubocurarine are repeated at hourly intervals throughout the course of the experiment. The subclavian arteries are occluded proximal to the vertebral origins. The right external carotid is occluded and two cannulae inserted into the right common carotid, one anterograde for measurement of Carotid Stump pressure (CSP) and one retrograde for measurement of systemic blood pressure. The right femoral artery is cannulated for arterial blood gas determinations. A midline scalp incision is made, and scalp and temporalis muscle are retracted laterally and retained by sutures around the zygomatic

arch. Bleeding points on the calvarium are controlled with electrocautery, bone wax, and painting the calvarium with acrylic.

The rat is maintained under anesthesia with ventilatory support and placed in a specially constructed probe containing plastic tubing coils for circulation of warm water for control of body temperature monitored with a rectal thermister, with a supplemental mouth thermister to enable control of any significant head-body temperature gradient. If necessary warm air can be circulated through the bore of the magnet and cool water through the coils for this purpose. The probe mounts a 1.5 cm one turn elliptical coil which is brought into contact with the calvarium and its position adjusted using skull landmarks.

EEG activity is recorded between a pair of platinum-iridium wire electrodes placed on the dural surface through small burr holes 2 mm diagonally apart on opposite sides of the midline at the approximate center of the coil. Care is taken to keep the wires as far as possible from the coil. Radiofrequency filters are interposed at the mouth of the magnet in order to exclude their effect as antennae which would introduce ambient electromagnetic radiation into the magnet. EEG thus acquired is amplified by a Grass Wide Band A.C. coupled EEG Pre-Amplifier. The output of the pre-amps is fed into Grass D.C. Driver Amplifiers, filtered at 15 Hz, the pen outputs multiplexed to an active EEG filter. The circuitry provides a quantitative measure of slow activity (0.3 to 5.6 Hz, maximum sensitivity 2Hz), and fast activity (4.4 to 15 Hz with 7 Hz maximum). The high frequency activity is particularly sensitive to metabolic impairment.

Control of brain and body temperatures is obtained by on-line monitoring of mouth and rectal temperatures and computer controlled pumping of warm water from a water bath to coils in a chamber surrounding the rats body. Occasionally magnet shimming produces so much heat in the magnet that is necessary to blow room air into the center of the magnet to permit adequate temperature control to be maintained.

Glucose: Direct spectrophotometric measurement of glucose from 10 ul samples of plasma obtained from hematocrit tubes permitted quantitative assessment of blood glucose (11), without excessive blood loss from the animal. Plasma glucose, hematocrit and arterial blood gases were determined immediately prior to the first episode of ischemia, and prior to sacrifice.

Successive episodes of total global ischemia and reperfusion were made by rapid inflation of an occluding cuff (Type OC-2A - In Vivo Metric, Healdsburg, CA) placed around the left carotid artery. Inflation and deflation are achieved within about 2 seconds by a computer controlled pump and valve system which also serves to maintain constant the duration and frequency of the ischemia-reperfusion cycles. Ischemias less than 20 seconds with reperfusions more than 40 seconds have had no cumulative effect on EEG. Recovery will be monitored for standard periods of 1 or 3 hours following the serial brief ischemias. The latter is the longest time it is practicable to continue MRS monitoring combined with blood pressure and brain perfusion control.

MRS methodology:

Elsewhere, Dr Hetherington developed a number of techniques for the quantitative study of brain metabolism using 1H MRS. These include the first application of semi-selective pulses to surface coil studies of brain metabolism, the development of spectral editing sequences (12), the derivation of a universal phase cycling scheme to eliminate phase artifacts in composite pulse experiments (13), application of these composite pulse techniques to surface coil studies of rat brain (14) to increase the S/N of the measurement by 40%, the development of composite pulse sequences which provide water suppression and S/N

increases of 50% for surface coil studies (15), heteronuclear editing sequences to allow the measurement of the turnover of amino acids in in-vivo rat brain (16) and the development of a phase cycling scheme to eliminate artifacts in spectral editing sequences and obtain lactate spectra from human forearm after exercise (17). These techniques were used to obtain quantitative measurements of lactate and glutamate in the proposed experiments.

1H MRS measurements were made using an ISIS localization sequence combined with water suppression and spectral editing techniques. Animal variability was minimized by acquiring the identical brain region in each experiment. The acquired volume was determined so as to include mainly cortex, but also be compatible with adequate time resolution. The spatial coordinates of the volume were be determined by standard MRI. Water suppression will be achieved by the combined use of a semi-selective pulses in a spin echo sequence with presaturation. Unambiguous measurement of the C-3 resonance of lactate, 1.33 ppm, the C-4 of glutamate, 2.33 ppm,and the C-2 of GABA, 1.9 ppm, will be obtained by use of spectral editing sequences using selective inversion pulses to their J-coupled partners at 4.11,2.09, and 2.3 ppm. Evolution delays of 68 ms, 34 ms, and 34 ms were used. Spectra were quantitated using creatine at 3.03 ppm as an internal standard. The chemical shift reference will also be verified by use of NAA as an internal chemical shift standard at 2.023 ppm. It is expected that edited spectra of lactate, glutamate, and GABA will be obtained serially with 2.5 min time resolution for changes in the 1H spectra.

31P MRS measurements were acquired using an ISIS volume localization sequence to insure that the identical brain region is studied in each experiment. The acquired volume will be identical to that observed in the 1H experiments. 31P MRS measurement were made using a pulse acquire sequence following the ISIS localization sequence. An external standard of HMPT will allow quantification. The phosphocreatine resonance at -2.35 ppm served as a chemical shift reference. 31P spectra were acquired by interleaving with 1H spectra, such that the 7.5 min required for 1H spectra provided adequate time to acquire a 31P spectrum of adequate S/N.

Quantitation studies and extract protocol: in vivo quantitation studies were carried out on tracheotomized and ventilated rats. T1 and T2 were measured for all metabolites. After performing quantitative measurements of lactate, GABA, glutamate, total creatine+PCr, (1H MRS), Pi, PCr, and ATP (31P MRS) the brain will be extracted for high resolution MRS analysis. The extracted brain volume will be equivalent to the approximate volume seen by the coil as predicted by computer simulation. The brain will be funnel frozen with liquid N2. The frozen tissue will then be extracted with a mix of methanol and perchloric acid. The extract will then be lyophilized and reconstituted in D2O at pH 7.4. Sodium 3-trimethylsilyl[2,2,3,3,2 H]-propionate (TSP) will be added as a concentration and chemical shift reference.

REFERENCES:

1. Boehme RJ, Conger KA, Anderson ML: Computer-Regulated constant pressure ischemia in the rat: the animal model. J Cereb Blood Flow Metab. 1988, 8:236-243.

2. Eke A, Strong E, Halsey JH Jr: Stability of Oxygen transmissibility during ischemia. Neurol Res 1981;3:211-228.

3. Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J Biol Chem 239, 18-30, 1964.

4. Lowry OH, Passonneau JV: The relationships between substrates and enzymes of glycolysis in the brain. J Biol Chem 239, 31-42, 1964.

5. Drewes LR, Gilboe DD: Glycolysis and the permeation of glucose and lactate in the isolated, perfused dog brain during anoxia and postanoxic recovery. J Biol Chem 248:2489-2496, 1973.

6. Pardridge WM, Connor JD, Crawford, IL: Permeability changes in the blood-brain barrier: causes and consequences. CRC Crit Rev Toxicol 3:159-199, 1975.

7. Pardridge W, Oldendorf W: Transport of metabolic substrates through the blood-brain barrier. J Neurochem 28:5-12, 1977.

8. Pollay M, Stevens FA: Starvation-induced changes in transport of ketone bodies across the blood-brain barrier. J Neurosci Res 5:163-172, 1980.

9. Gjedde A, Crone C: Induction processes in blood-brain transfer of ketone bodies during starvation. Am J Physiol 229;1165-1169, 1975.

10. Moore TJ, Lione AP, Sugden MC, Regen, DM: Beta-hydroxybutyrate transport in rat brain: developmental and dietary modulations. Am J Physiol 230:619-630, 1976.

11. Lowry OH, Passonneau JV: A flexible system of enzymatic analysis. 1972 Academic Press, New York. pp. 174-177.

13. Hetherington HP and Rothman DL: Phase cycling of composite refocusing pulses to eliminate dispersive refocusing magnetization. J Magn Reson 1985;65:348.

14. Hetherington HP, Wishart D, Fitzpatrick SM, Cole P, Shulman RG: The application of composite pulses to surface coil NMR. J Magn Reson 1986;66:313.

15. Hetherington HP, PhD Thesis Chapter 5.

16. Fitzpatrick SM, Hetherington HP, Behar KL, Shulman RG: The flux from glucose to glutamate in the rat brain in vivo as determined by 1H-observer, 13C-edited NMR spectroscopy. J CBF Metabol 1990;10:170-179.

17. Hetherington HP, Hamm JR, Pan JW, Rothman DL, Shulman RG: Fully localized homonuclear edited ^{1}H NMR spectra of lactate in the human arm after exercise. J Magn Reson 1989;82:86.